Pergamon

Tetrahedron, Vol. 53, No. 25, pp. 8629-8634, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00532-2

Synthesis of the Unique Terminal Branched Tetrasaccharide of *Mycobacterium gordonae* Strain 990

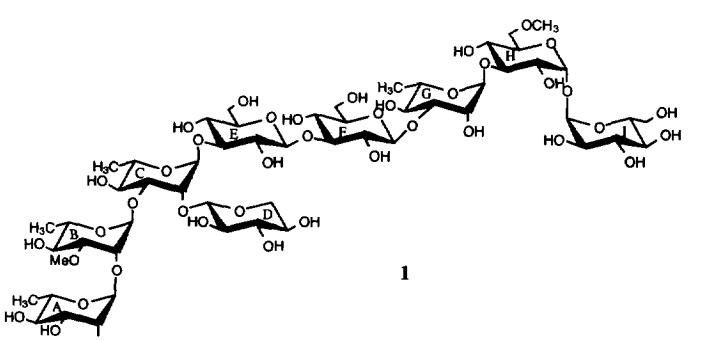
Mukund K Gurjar* and Susanta Adhikari

Indian Institute of Chemical Technology, Hyderabad 500 007, India

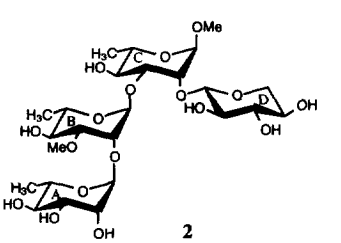
Abstract: *Mycobacterium gordonae* strain 990 contains a unique oligosaccharide with branched sugar residue. A novel synthesis of the terminal tetrasaccharide-methyl 3-O-[3-O-methyl-2-O(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-2-O-(β-D-xylopyranosyl)-α-L-rhamnopyranoside has been described.

Since the outbreak of acquired immunodefficiency syndrome (AIDS), the importance of mycobacterial diseases has been revived.[1] The occurrence of diseases due to mycobacteria, apart from tuberculosis, are on the rise particularly in patients with AIDS. Nontuberculotic mycobacteria contain a large amount of glycolipids, whose carbohydrate segment is associated with antigenic activity. More importantly, the terminal oligosaccharides possess the most antigenic activity and therefore investigations involving the synthesis of the terminal oligosaccharide has been targeted in order to evoke corresponding antibodies, necessary for serodiagnosis[2] of individual mycobacteria.[3]

Mycobacterium gordonae strain 990 belongs to trehalose containing lipooligosaccharide. Infections due to *Mycobacterium (M.) gordonae* are reported involving skin and soft tissues, trauma and underlying immunosuppression. The strain is observed in patients with advanced immunodefficiency causing pulmonary infections which are indistinguishable from that of mycobacterial tuberculosis. Patients are given classical chemotherapy for tuberculosis which include drugs such as isoniazid, pyrazinamide, ethambutol and cycloserine, however, *M. gordonae* is resistant to them. It is, therefore, essential to diagnose atypical mycobacteria in order to provide the required chemotherapy.



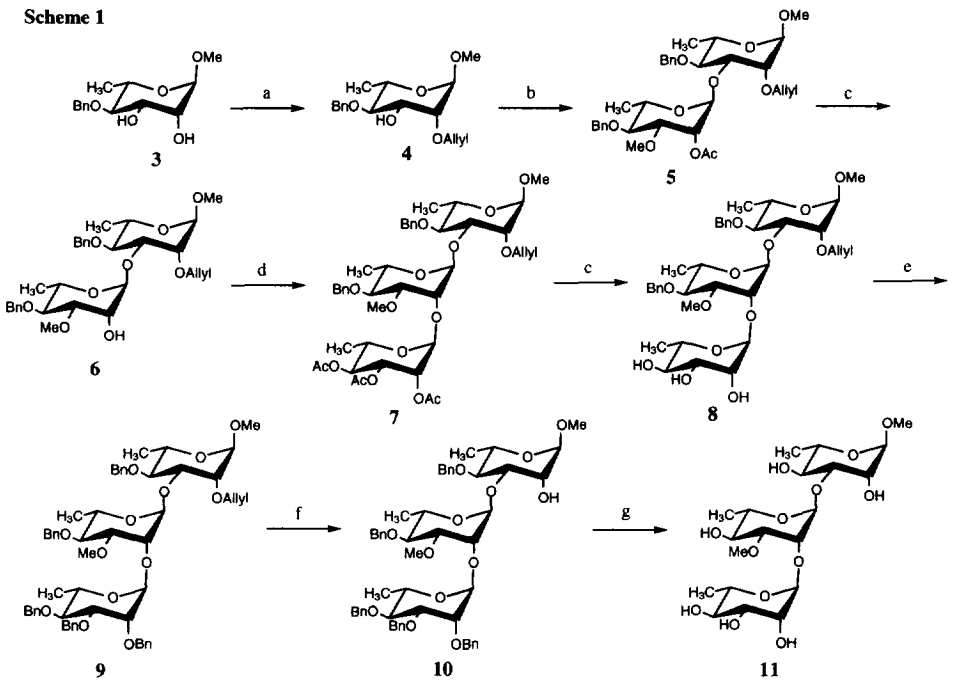
1



2

Structural elucidation[4] of glycolipid of mycobacteria provided an element of surprise and uniqueness. The oligosaccharide segment **1** of *M. gordonae* (strain 990) has a unique structural feature namely the presence of branched sugar β-D-xylopyranosyl residue linked to O-2 position of L-rhamnose unit (ring C) of linear oligosaccharide structure. In this report we describe the first synthesis of the terminal tetrasaccharide subunit (A-D) represented by the structure **2**.

Scheme 1



Reagents and Conditions: (a) AllylBr, 5% NaOH, Bu_4NBr, CH_2Cl_2, RT, 6h; (b) 2-O-Ac-4-O-Bn-3-O-Me-L-Rhap-O-C(CCl_3)=NH, BF_3:OEt_2, 4A Mol. sieves, CH_2Cl_2, -20°C-RT, 3h; (c) NaOMe, MeOH, RT, 1h; (d) 2,3,4-($OAc)_3$-L-Rhap-O-C($CCl)_3$=NH; BF_3:OEt_2, 4A Mol. sieves, CH_2Cl_2, -20°C-RT, 3h; (e) BnBr, NaH, THF, RT, 12h; (f) SeO_2, AcOH, dioxane, 90°C, 1h; (g) 10% $Pd(OH)_2$-C, H_2, 1 atm, MeOH, 48h.

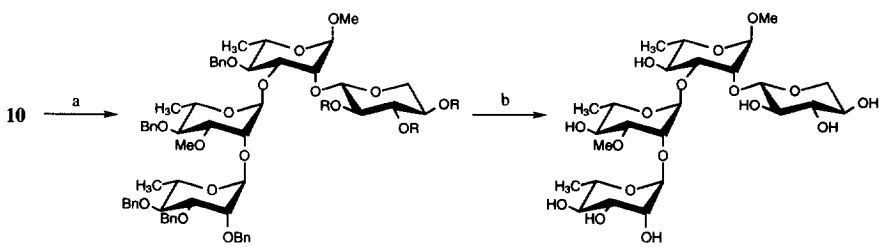
Methyl 2-O-allyl-4-O-benzyl-α-L-rhamnopyranoside **4** was synthesised from methyl 4-O-benzyl-α-L-rhamnopyranoside under phase transfer condition[5] by using allyl bromide, 5% NaOH solution and tetrabutylammonium bromide in CH_2Cl_2 at room temperature. The ^1H NMR spectrum and optical rotation of **4** were compared with the corresponding methyl 3-0-allyl-4-O-benzyl-α-L-rhamnopyranoside, prepared earlier in our laboratory[6] and the structure of **4** was assigned. The glycosylating agent 2-O-acetyl-4-O-benzyl-3-O-methyl-L-rhamnopyranosyl-trichloroacetimidate was earlier synthesised in these laboratories[7] from L-rhamnose. Its subsequent condensation with **4** was promoted[8] by BF_3:OEt_2 at -20°C to give rise to the disaccharide **5**. In the ^1H NMR spectrum of **5**, the anomeric protons were located at 4.79 and 4.96 ppm as singlets. The remaining signals were in complete agreement with the assigned structure . Treatment of **5** with methanolic sodium methoxide provided **6** with the hydroxy group at C-2 free for further glycosidation. In order to confirm the structure of **6**, partially decoupled ^{13}C NMR spectrum was recorded. The characteristic

coupling constants ($J_{C,H}$ = 167 and 172 Hz) for anomeric carbons confirmed α configurations at C-1 and C-1' of **6**. The ^1H NMR spectrum of **6** revealed the expected absence of an acetyl singlet at 2.09 ppm while the anomeric protons were localised at 4.75 and 5.07 ppm as singlets.

The O-glycosidation of aglycone **6** with 2,3,4-tri-O-acetyl-L-rhamnopyranosyl-trichloroacetimidate[6] in the presence of BF_3:OEt_2 at -20°C gave the trisaccharide **7**, the acetyl groups of which were removed by Zemplen deacetylation. The ^1H NMR spectrum of **8** showed three doublets at 1.11, 1.18 and 1.25 ppm corresponding to 5-CH_3, 5'-CH_3 and 5"-CH_3. The anomeric protons were clearly observed at 4.84, 4.91 and 5.00 ppm as singlets. Compound **8** was benzylated in the presence of NaH and BnBr to give the penta-O-benzylate derivative **9**. The α-configurations of anomeric carbons of **9** were confirmed by the partially decoupled ^{13}C NMR spectrum in which the values of coupling constants for anomeric carbons ($J_{C,H}$= 173, 170 and 167 Hz) were found above 165 Hz. Our next concern was the removal of allyl group at O-2 for which compound **9** was treated[9] with SeO_2-HOAc to give rise to **10**. Compound **10** was exhaustively debenzylated using 10% $Pd(OH)_2$-C in methanol under hydrogen at normal temperature and pressure to give the trisaccharide methyl 3-O-[3-O-methyl-2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside **11** (Scheme 1).

In order to synthesise the tetrasaccharide **2**, compound **10** having a free C-2 hydroxyl, was treated with 2,3,4-tri-O-acetyl-α-D-xylopyranosyl bromide in the presence of $HgBr_2$ and $Hg(CN)_2$ at 0°C to give

Scheme 2



12 R = Ac
13 R = H

2

Reagents and Conditions: (a)(i) 2,3,4-$(OAc)_3$-D-Xylop-Br, $Hg(CN)_2$, $HgBr_2$, CH_2Cl_2, RT, 12h; (ii) NaOMe, MeOH, RT, 1h; (b) 10% $Pd(OH)_2$-C, H_2, 1 atm, MeOH, 48h.

the crude tetrasaccharide **12** which was, however, contaminated with small quantities of 2,3,4-tri-O-acetyl-xylose. The mixture was deacetylated under Zemplen conditions and then chromatographed on silica gel to give pure tetrasaccharide **13**. The ^1H NMR spectrum of tetrasaccharide showed the characteristic doublet (J = 7.34 Hz) at 4.38 ppm for anomeric proton which corresponded with β-configuration of xylopyranosyl residue. The hydrogenolysis of **13** in the presence of 10% $Pd(OH)_2$-C in methanol under hydrogen at normal temperature and pressure gave the final product **2**. The ^1H NMR spectrum of **2** showed three singlets at δ 4.59, 4.76 and 5.14 corresponding to three α-rhamnosyl residues and a doublet at δ 4.39 (J = 7.6 Hz) due to β-D-xylopyranosyl sugar (Scheme 2).

Thus, we have demonstrated a practical approach for the synthesis of unique terminal tetrasaccharide segment of *M. gordonae*.

Experimental

Methyl 2-O-allyl-4-O-benzyl-α-L-rhamnopyranoside (4)

To a solution of **3** (1.0 g, 3.7 mmol) and allyl bromide (0.54 g, 4.5 mmol) in CH_2Cl_2 (10 mL) was added 5% NaOH solution (10 mL) and tetrabutylammonium bromide (0.38g, 0.9 mmol). The reaction mixture was stirred at room temperature for 6 h and then the organic layer separated, washed with brine, dried over Na_2SO_4 and concentrated. The residue was chromatographed on silica gel using ethyl acetate - light petroleum (1:15) as eluent to give **4** (0.92 g, 80%), as a syrup, $[\alpha]_D$ -38° (c 1.5, chloroform); ^1H NMR (200 MHz, $CDCl_3$) : δ 1.29 (d, 3 H, J = 5.7 Hz), 3.22 (t, 1 H, J = 9.0 Hz), 3.34 (s, 3 H), 3.59 (m, 2 H), 3.88 (dd, 1 H, J = 4.5, 9.0 Hz), 4.12 (m, 2 H), 4.63 (s, 1 H), 4.65, 4.90 (2d, 2 H, ABq, J = 11.4 Hz), 5.15 -5.4 (m, 2 H), 5.90 (m, 1 H), 7.30 (m, 5 H); HRFABMS : $C_{17}H_{24}O_5$ $[M]^+$, calcd.: m/z = 308.1623, found 308.1629 (error 2.0 ppm).

Methyl 2-O-allyl-4-O-benzyl-3-O-(4-O-benzyl-3-O-methyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (6)

To a stirred solution of **4** (0.22 g, 0.7 mmol), 2-O-acetyl-4-O-benzyl-3-O-methyl-L-rhamnopyranosyl-trichloroacetimidate (0.33 g, 0.7 mmol) and powdered 4A molecular sieves (1 g) in CH_2Cl_2 (10 mL) at -20°C was added freshly distilled BF_3:OEt_2 (67 μL, 0.54 mmol). After 3 h at room temperature, the reaction was quenched with triethylamine (0.3 mL) and filtered. The filtrate was washed with water, dried over Na_2SO_4, concentrated and the residue chromatographed on silica gel by eluting with ethyl acetate - light petroleum (1:25) to give **5** (0.40 g, 90%), $[\alpha]_D$ -39° (c 1, chloroform); ^1H NMR (200 MHz, $CDCl_3$) : δ 1.28 (d, 6 H, J = 6.5 Hz), 2.09 (s, 3 H), 3.29 (t, 1 H, J = 9.0 Hz), 3.34, 3.36 (2s, 6 H), 3.4 - 3.7 (m, 5 H), 3.75 - 4.05 (m, 2 H), 4.15 (m, 2 H), 4.61 (m, 3 H), 4.79 (s, 1 H), 4.88 (1/2. ABq, 1 H, J = 11.3 Hz), 4.96 (s, 1 H), 5.2 (m, 2 H), 5.38 (bs, 1 H), 5.9 (m, 1 H), 7.3 (m, 10 H); FABMS : m/z 601 $[M+H]^+$.

The above compound **5** (0.4 g, 0.7 mmol) was dissolved in methanol (5 mL) and sodium metal (30 mg) added. After 1 h the reaction was deionised with Amberlite IR 120 (H) resin, filtered, concentrated and purified on silica gel by using ethyl acetate - light petroleum (1:8) as eluent to give **6** (0.35 g, 95%), $[\alpha]_D$ -59° (c 1.5, chloroform); ^1H NMR (200 MHz, $CDCl_3$) : δ 1.25, 1.28 (2d, 6 H, J = 6.8 Hz), 3.34, 3.47 (2s, 6 H), 3.3 - 3.7 (m, 5 H), 3.85 (m, 1 H), 3.95 (m, 2 H), 4.15 (m, 2 H), 4.6 (m, 3 H), 4.75 (s, 1 H), 4.81 (1/2. ABq, 1 H, J = 11.3 Hz), 5.07 (s, 1 H), 5.1 - 5.4 (m, 2 H), 5.9 (m, 1 H), 7.3 (m, 10 H). ^{13}C NMR (200 MHz, $CDCl_3$) : δ 98.60 (J = 167 Hz), 100.99 (J = 172 Hz); HRFABMS: $C_{31}H_{41}O_9$ $[M-H]^+$, calcd.: m/z = 557.2756, found 557.2750 (error 1.0 ppm).

Methyl 2-O-allyl-4-O-benzyl-3-O-[4-O-benzyl-3-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (9)

To a stirred solution of **6** (0.35 g, 0.6 mmol), 2,3,4-tri-O-acetyl-L-rhamnopyranosyl-trichloroacetimidate (0.27 g, 0.6 mmol) and powdered 4A molecular sieves (1 g) in CH_2Cl_2 (10 mL) at -20°C was added freshly distilled BF_3:OEt_2 (58 μL, 0.47 mmol). After 3 h at room temperature the reaction mixture was quenched with triethylamine (0.3 mL) and filtered. The filtrate was washed with water, dried over Na_2SO_4 and concentrated to provide the crude product which was purified on silica gel by eluting with ethyl acetate - light petroleum (1:7) to give **7** (0.44 g, 85%), $[\alpha]_D$ -49° (c 2, chloroform); FABMS : m/z = 830 $[M]^+$.

The above compound **7** (0.44 g, 0.5 mmol) was deacetylated by using methanolic sodium methoxide (74 mg of Na in 5 mL of methanol). After 1h, the reaction mixture was deionised with Amberlite IR 120 (H) resin, filtered, concentrated to give the crude product **8** which was isolated after chromatography, $[\alpha]_D$ -53° (c 2, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.11. 1.18, 1.25 (3d, 9 H, J = 6.5 Hz), 3.27, 3.29 (2s, 6 H), 3.40 (t, 1 H, J = 9.0 Hz), 3.5 - 4.3 (m, 13 H), 4.54, 4.81 (ABq, 2 H, J = 11.7 Hz), 4.57 (s, 2 H), 4.84 (s, 1 H), 4.91 (s, 1 H), 5.00 (s, 1 H), 5.1 - 5.35 (m, 2 H), 5.9 (m, 1 H), 7.3 (bs, 10 H).

The compound **8** (0.34 g, 0.5 mmol) was dissolved in dry THF (7 mL) and sodium hydride (0.4 g, 60% dispersion in oil) introduced. After stirring for 1 h, benzyl bromide (0.5 mL, 4.2 mmol) was added. The reaction mixture was stirred for 12 h, excess of sodium hydride was decomposed by methanol and then concentrated. The residue was partitioned between ethyl acetate - water and the organic layer was dried over Na_2SO_4, concentrated and chromatographed on silica gel by eluting with ethyl acetate - light petroleum (1:12) to give **9** (0.4 g, 85%), $[\alpha]_D$ -51° (c 1, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.2 (m, 9 H), 3.18 (t, 1 H, J = 9.0 Hz), 3.31, 3.34 (2s, 6 H), 3.45 (t, 1 H, J = 9.0 Hz), 3.5 - 4.2 (m, 12 H), 4.5, 5.4 (m, 15 H), 5.9 (m, 1 H), 7.3 (bs, 25 H). ^{13}C NMR (200 MHz, $CDCl_3$) : δ 98.29 (J = 170 Hz), 99.36 (J = 167 Hz), 100.98 (J = 173 Hz); HRFABMS: $C_{58}H_{69}O_{13}$ $[M-H]^+$, calcd.: m/z = 973.5677, found 973.5696 (error 2.0 ppm).

Methyl 4-O-benzyl-3-O-[4-O-benzyl-3-O-methyl-2-O-(2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (10).

Compound **9** (0.34 g, 0.34 mmol), SeO_2 (0.04 g, 0.38 mmol) and acetic acid (30 μL) in dioxane (10 mL) were heated at 90°C for 1 h, filtered and concentrated. The residue was chromatographed on silica gel using ethyl acetate - light petroleum (1:10) to give **10** (0.242 g, 75%). $[\alpha]_D$ -55° (c 1, chloroform); ^{1}H NMR (200 MHz, $CDCl_3$) : δ 1.25 (m, 9 H), 3.20 (t, 1 H, J = 9.0 Hz), 3.32, 3.34 (2s, 6 H), 3.38 (t, 1 H, J = 9.0 Hz), 3.45 - 4.0 (m, 10 H), 4.5 - 4.65 (m, 6 H), 4.67 - 4.85 (m, 5 H), 4.93 (s, 1 H), 5.00 (s, 1 H), 7.3 (bs, 25 H); HRFABMS: $C_{55}H_{67}O_{13}$ $[M+H]^+$, calcd.: m/z = 935.5520, found 935.5559 (error 4.2 ppm).

Methyl 3-O-[3-O-methyl-2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-α-L-rhamnopyranoside (11).

Compound **10** (0.2 g, 0.21 mmol) and 10% $Pd(OH)_2$-C (50 mg) in methanol (10 mL), was hydrogenated at normal temperature and pressure for 48 h. The catalyst was filtered and the filtrate concentrated. The residue was purified by column chromatography on silica gel by eluting with chloroform - methanol (10:1) to give **11** (96 mg, 95%), $[\alpha]_D$ -45° (c 0.6, methanol); ^{1}H NMR (400 MHz, CD_3OD) : δ 4.34 (s, 1 H), 4.94 (s, 1 H), one anomeric signal merged with HOD signal at δ 4.70.

HRFABMS : $C_{20}H_{37}O_{13}$ $[M+H]^+$, calcd.: m/z = 485.2273, found 485.2234 (error 8.0 ppm).

Methyl 3-O-[3-O-methyl-2-O-(α-L-rhamnopyranosyl)-α-L-rhamnopyranosyl]-2-O-(β-D-xylopyranosyl)-α-L-rhamnopyranoside (2).

To a stirred solution of **10** (0.24 g, 0.26 mmol), $Hg(CN)_2$ (60 mg), $HgBr_2$ (23 mg) and 4A molecular sieves (1 g) in CH_2Cl_2 (10 mL) was added 2,3,4-tri-O-acetyl-D-xylopyranosyl bromide (0.18 g, 0.5 mmol). After 12 h at room temperature the reaction mixture was worked-up and the residue purified by silica gel column chromatography using ethyl acetate - light petroleum (1:12) to give **12** (0.18 g) which was contaminated with 2,3,4-tri-O-acetyl-xylopyranose.

The above crude product **12** was deacetylated by using methanolic sodium methoxide (18 mg of sodium in 5 mL of methanol) for 1h.The reaction mixture was neutralised with Amberlite IR 120 (H) resin, filtered and concentrated, followed by chromatography on silica gel by using ethyl acetate - light petroleum (1:2) to give **13** (0.14 g, 51%), $[\alpha]_D$ -46° (c 0.5, chloroform); ^{1}H NMR (400 MHz, $CDCl_3$) : δ 1.10 (d, 3 H, J = 6.5 Hz), 1.17 (m, 6 H), 3.12 (t, 1 H, J = 9.0 Hz), 3.23 (t, 1 H, J = 9.0 Hz), 3.24 (s, 3 H), 3.27 (s, 3 H), 3.27 - 3.7 (m, 9 H), 3.76 (m, 2 H), 3.82 - 3.98 (m, 3 H), 4.38 (d, 1 H, J = 7.34 Hz), 4.46 (1/2, ABq, 1 H), 4.47 (1/2, ABq, 1 H), 4.53 (ABq, 2 H), 4.54 (1/2, ABq, 1 H), 4.62 (d, 1 H, J = 1.8 Hz), 4.65 (s, 2 H), 4.71 (1/2, ABq, 2 H), 4.83 (1/2, ABq, 1 H), 4.89 (d, 1 H, J = 1.0 Hz), 4.97 (d, 1 H, J = 1.5 Hz), 7.3 (m, 25 H); FABMS : m/z = 1066 $[M]^+$.

Compound **13** (75 mg, 0.07 mmol) and 10% $Pd(OH)_2$-C (20 mg) in methanol (5 mL) was stirred under hydrogen at normal temperature and pressure for 48 h. The catalyst was filtered and the residue was concentrated to give **2** (34 mg, 80%), $[\alpha]_D$ -43° (c 0.5, methanol); ^{1}H NMR (400 MHz, CD_3OD) : δ 1.2 (m, 9 H), 3.1-3.65 (m, 12 H), 3.24, 3.29 (2s, 6 H), 3.75 (m, 4 H), 4.12 (m, 1 H), 4.39 (d, 1 H, J = 7.6 Hz), 4.59 (s, 1 H), 4.76 (s, 1 H), 5.14 (s, 1 H); HRFABMS : $C_{25}H_{45}O_{17}$ $[M+H]^+$, calcd.: m/z = 617.2651, found 617.2656 (error 0.9 ppm).

Acknowledgement: One of us (S. Adhikari) is greatful to CSIR, New Delhi for awarding JRF.

References:

1. (a) Aspinall, G.O.; Chatterjee, D.; Brennan, P.J. *Adv. Carbohydr. Chem. Biochem.* **1995**, 51, 169. (b) Brennan, P.J.; *Microbial lipids*. (Ratledge, C.;Wilkinson, S.G.W Eds),*Academic Press, London.* **1988**, 1, 251.
2. Hunter, S.W.; Jardine, I.; Yanagihara, D.L.; Brennan, P.J. *Biochem.* **1985**, 24, 2798.
3. Gaylord, H.; Brennan, P.J. *Annu. Rev. Microbiol.* **1987**, 41, 645.
4. Basra, G.S.; McNeil, M.R.; Khoo, K-H.; Bell, A.; Morris, H.R.; Brennan, P.J. *Biochem.* **1993**, 32, 12705.
5. Garegg, P.J.; Iversen, T.; Oscarson, S. *Carbohydr. Res.* **1976**, 50, C-12.
6. Gurjar, M.K.; Mainkar, A.S. *Tetrahedron.* **1992**, 48, 6729.
7. Gurjar, M.K.; Saha, U.K. *Bio. Org. Med. Chem. Lett.* **1993**, 3, 697.
8. Schmidt, R.R. *Angew. Chem. Int. Ed. Engl.* **1986**, 25, 212.
9. Kariyone, K and Yazawa, H, *Tetrahedron Lett.* **1970**, 2885.

IICT Communication No. 3796

(*Received in UK* 10 *March* 1997; *accepted* 15 *May* 1997)